STATE OF MISSOURI

DEPARTMENT OF NATURAL RESOURCES

MISSOURI CLEAN WATER COMMISSION

MISSOURI STATE OPERATING PERMIT

In compliance with the Missouri Clean Water Law, (Chapter 644 R.S. Mo. as amended, hereinafter, the Law), and the Federal Water Pollution Control Act (Public Law 92-500, 92nd Congress) as amended,

Permit No.:	MO-0095362
Owner:	Empire District Electric Company
Address:	PO Box 127, Joplin, MO 64802
Continuing Authority:	Same as above
Address:	Same as above
Facility Name:	Empire District Electric Company, Asbury Plant
Address:	21133 Uphill Lane, Asbury, MO 64832
Legal Description:	Outfalls #001-#003 - NE ¼, NW ¼, Sec. 17, T30N, R33W, Jasper Co. Outfall #004 - SE ¼, SW ¼, Sec. 8, T30N, R33W, Barton Co.
Receiving Stream:	Blackberry Creek (U)
First Classified Stream and ID:	Blackberry Creek (C)(03184)
USGS Basin & Sub-watershed No.:	(11070207-140003)

is authorized to discharge from the facility described herein, in accordance with the effluent limitations and monitoring requirements as set forth herein:

FACILITY DESCRIPTION

See page 2

This permit authorizes only wastewater discharges under the Missouri Clean Water Law and the National Pollutant Discharge Elimination System; it does not apply to other regulated areas. This permit may be appealed in accordance with Section 644.051.6 of the Law.

April 21, 2000
Effective Date

February 6, 2004
Revised

Stephen M. Mahfood, Director, Department of Natural Resources
Executive Secretary, Clean Water Commission

April 20, 2005
Expiration Date

Jim Hull, Director of Staff, Clean Water Commission

MO 780-0041 (10-93)

FACILITY DESCRIPTION (continued)

Outfall #001 - Industry - SIC #4911
Cooling water discharge.
Design flow is 1,000,000 gallons per day.
Actual flow is 720,000 gallons per day.

Outfall #002 - Industry - SIC #4911
Ash pond overflow.

Outfall #003 – Industry - SIC #4911
Storm water runoff.
Actual flow is dependent upon precipitation.

Instream Monitoring - Industry - SIC #4911
Upstream and downstream monitoring.

Outfall #004 – Sewage Treatment – SIC #4952
Extended aeration/single cell lagoon/sludge is retained in lagoon.
*Was previously covered under NPDES Permit #MO-0106381
Design population equivalent is 80.
Design flow is 8,000 gallons per day.
Actual flow is 2,000 gallons per day.
Design sludge production is 1.4 dry tons/year.

A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The interim effluent limitations shall become effective upon issuance and remain in effect until December 31, 2002. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	INTERIM EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
		DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Outfall #002						
Flow	MGD	*		*	once/day	24 hr. total
Total Suspended Solids	mg/L	100		30	once/month	grab
Oil and Grease	mg/L	20		15	once/month	grab
pH – Units	SU	**		**	once/month	grab
Sulfate as SO_4	mg/L	*		*	once/month	grab
Iron, Total Recoverable	mg/L	1.0		1.0	once/month	grab
Copper, Total Recoverable	mg/L	0.041		0.041	once/month	grab
Ammonia as N	mg/L	5.0		5.0	once/month	grab
MONITORING REPORTS SHALL BE SUBMITTED MONTHLY; THE FIRST REPORT IS DUE March 28, 2004.						
Whole Effluent Toxicity (WET) Test	% Survival	See Special Condition #14			once/year	grab
MONITORING REPORTS SHALL BE SUBMITTED ANNUALLY; THE FIRST REPORT IS DUE October 28, 2004.						
Outfall #003 (Note 1)						
Flow	cfs	*		*	once/quarter***	instantaneous estimate
pH – Units	SU	**		**	once/quarter***	grab
Ammonia as N	mg/L	5.0		5.0	once/quarter***	grab
Sulfate as SO_4	mg/L	*		*	once/quarter***	grab
Temperature	°F	*		*	once/quarter***	grab
Iron, Total Recoverable	mg/L	*		*	once/quarter***	grab
Copper, Total Recoverable	mg/L	*		*	once/quarter***	grab
MONITORING REPORTS SHALL BE SUBMITTED QUARTERLY; THE FIRST REPORT IS DUE April 28, 2004. THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.						

B. STANDARD CONDITIONS

IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED Part I STANDARD CONDITIONS DATED October 1, 1980, AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.

A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
		DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Outfall #001						
Flow	MGD	*		*	once/day	24 hr. total
Temperature	°F	90°		90°	once/week	grab
Temperature Increase	°F	5°		5°	once/week	grab
Chlorine, Free Available	mg/L	0.5		0.2	once/month	grab
pH – Units	SU	**		**	once/month	grab
Ammonia as N	mg/L	5.0		5.0	once/month	grab

MONITORING REPORTS SHALL BE SUBMITTED MONTHLY; THE FIRST REPORT IS DUE March 28, 2004. THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.

B. STANDARD CONDITIONS

IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED Part I STANDARD CONDITIONS DATED October 1, 1980, AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.

A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective January 1, 2003 and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
		DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Outfall #002						
Flow	MGD	*		*	once/day	24 hr. total
Total Suspended Solids	mg/L	100		30	once/month	grab
Oil and Grease	mg/L	20		15	once/month	grab
pH – Units	SU	**		**	once/month	grab
Sulfate as SO_4	mg/L	1000		1000	once/month	grab
Iron, Total Recoverable	mg/L	1.0		1.0	once/month	grab
Copper, Total Recoverable	mg/L	0.041		0.041	once/month	grab
Ammonia as N	mg/L	5.0		5.0	once/month	grab
MONITORING REPORTS SHALL BE SUBMITTED MONTHLY; THE FIRST REPORT IS DUE March 28, 2004.						
Whole Effluent Toxicity (WET) Test	% Survival	See Special Conditions			once/year	grab
MONITORING REPORTS SHALL BE SUBMITTED ANNUALLY; THE FIRST REPORT IS DUE October 28, 2004.						
Outfall #003 (Note 1)						
Flow	cfs	*		*	once/quarter***	instantaneous estimate
pH – Units	SU	**		**	once/quarter***	grab
Ammonia as N	mg/L	5.0		5.0	once/quarter***	grab
Sulfate as SO_4	mg/L	1000		1000	once/quarter***	grab
Temperature	°F	*		*	once/quarter***	grab
Iron, Total Recoverable	mg/L	*		*	once/quarter***	grab
Copper, Total Recoverable	mg/L	*		*	once/quarter***	grab
MONITORING REPORTS SHALL BE SUBMITTED QUARTERLY; THE FIRST REPORT IS DUE April 28, 2004. THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.						

B. STANDARD CONDITIONS

IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED Part I STANDARD CONDITIONS DATED October 1, 1980, AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.

A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
		DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Outfall #004						
Flow	MGD	*		*	once/quarter***	24 hr. estimate
Biochemical Oxygen Demand$_5$	mg/L		45	30	once/quarter***	grab
Total Suspended Solids	mg/L		45	30	once/quarter***	grab
pH – Units	SU	**		**	once/quarter***	grab

MONITORING REPORTS SHALL BE SUBMITTED QUARTERLY; THE FIRST REPORT IS DUE April 28, 2004. THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.

B. STANDARD CONDITIONS

IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED Parts I & III STANDARD CONDITIONS DATED October 1, 1980 and August 15, 1994, AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.

A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
		DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Instream Monitoring Point (Upstream of Outfall #001)						
Flow	cfs	*			once/month	instantaneous estimate
pH – Units	SU	**			once/month	grab
Iron, Total Recoverable	mg/L	*			once/month	grab
Copper, Total Recoverable	mg/L	*			once/month	grab
Ammonia as N	mg/L	*			once/month	grab
Sulfate as SO_4	mg/L	*			once/month	grab
Temperature	°F	*			once/month	grab
Instream Monitoring Point (Downstream of Outfall #002) (Note 2)						
Flow	cfs	*			once/month	instantaneous estimate
pH – Units	SU	**			once/month	grab
Iron, Total Recoverable	mg/L	*			once/month	grab
Copper, Total Recoverable	mg/L	*			once/month	grab
Ammonia as N	mg/L	*			once/month	grab
Sulfate as SO_4	mg/L	*			once/month	grab
Temperature Increase	°F	5°F			once/month	grab

MONITORING REPORTS SHALL BE SUBMITTED MONTHLY; THE FIRST REPORT IS DUE March 28, 2004. THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.

B. STANDARD CONDITIONS

IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED Part I STANDARD CONDITIONS DATED October 1, 1980, AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.

A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS (continued)

* Monitoring requirement only.
** pH is measured in pH units and is not to be averaged. The pH is limited to the range of 6.0-9.0 pH units.
*** Sample once per quarter in the months of March, June, September, and December.

Note 1 – Samples shall be taken during the first hour of a stormwater runoff event near the north branch location.

Note 2 – Samples shall be taken at the bridge on County Road 290 immediately southeast of the Asbury Plant property boundaries; notated as north bridge on permit application. If NH_3 exceeds 5.0 mg/L at this point, the Department of Natural Resources Water Pollution Control Program must be notified in writing within five days. Another monitoring point must be established at Highway H, where the classified section of the stream begins.

C. SPECIAL CONDITIONS

1. This permit may be reopened and modified or alternatively revoked and reissued, to incorporate new or modified effluent limitations or other conditions, if the result of a wasteload allocation study, toxicity test, or other information indicates changes are necessary to assure compliance with Missouri's Water Quality Standards.

2. Permittee shall continue to investigate disposal options for accumulated power plant wastes currently being stored in ash ponds on the plant site. An annual report on progress towards ultimate disposal of these wastes should be sent to the Springfield Regional Office prior to July 1 of each year.

3. This permit authorizes the continued use of existing or new storm sewers to convey uncontaminated storm runoff. Such uncontaminated outfalls do not require monitoring or limitations.

4. Permittee shall submit an annual report on the ash pond seeps, including, but not limited to:

   (a) an estimate of the minimum and maximum flow rates of all seeps combined;
   (b) any historical changes observed in flow rates;
   (c) the number of seeps observed and the quality of water in the seeps;
   (d) comments on any new seeps that occur in the berms or diking;
   (e) the permittee's own evaluation of the impact of the seeps on the quality of the receiving waters.

   The annual report is to be submitted to the Southwest Regional Office prior to July 1 of each year, along with the annual report on disposal of power plant wastes.

5. There shall be no discharge of polychlorinated bipheyl compounds.

6. Discharge of wastewater from this facility must not alone or in combination with other sources cause the receiving stream to violate the following:

   (a) Water temperatures and temperature differentials specified in Missouri Water Quality Standards shall be met.

7. Any pesticide discharge from any point source shall comply with the requirements of the Federal Insecticide, Fungicide and Rodenticide Act, as amended (7 U.S.C. 136 et seq.) and the use of such pesticides shall be in a manner consistent with its label.

8. Except for any untreated overflow from facilities designed, constructed and operated to treat the volume of material storage runoff which is associated with a 10 year, 24 hour rainfall event; discharges resulting from material storage runoff shall comply with the following limitation:

   (a) Total suspended solids shall not exceed 50 mg/l at any time.
   (b) The pH shall not be less the 6.0 standard units nor greater than 9.0 standard units at any time.

9. Neither free available chlorine nor total residual chlorine may be discharged from any unit for more than two hours in any one day and not more than one unit in any plant may discharge free available or total residual chlorine at any one time.

10. Outfall #003 shall be sampled at a point which includes all stormwater discharges from the railroad loop area but which is prior to confluence with the north branch. Permittee shall establish a permanent marker to show where sampling is taking place.

C. SPECIAL CONDITIONS (continued)

11. Discharges shall not cause violations of the general criteria in the Water Quality Standards 10 CSR 20-7.031 (3), which states, in part, that no water contaminant, by itself or in combination with other substances, shall prevent the waters of the State from meeting the following conditions:

(a) Waters shall be free from substances in sufficient amounts to cause the formation of putrescent, unsightly or harmful bottom deposits or prevent full maintenance of beneficial uses;
(b) Waters shall be free from oil, scum and floating debris in sufficient amounts to be unsightly or prevent full maintenance of beneficial uses;
(c) Waters shall be free from substances in sufficient amounts to cause unsightly color or turbidity, offensive odor or prevent full maintenance of beneficial uses; and
(d) Waters shall be free from substances or conditions in sufficient amounts to have a harmful effect on human, animal or aquatic life.

12. Report as no-discharge when a discharge does not occur during the report period.

13. Treatment or Storage of Ash from Power Plants

(a) Disposal of ash is not authorized by this permit.
(b) This permit does not pertain to permits for disposal of ash or exemptions for beneficial uses of ash under the Missouri Solid Waste Management Law and regulations.
(c) This permit does not authorize off-site storage, use or disposal of ash in regard to water pollution control permits required under 10 CSR 20-6.015 and 10 CSR 20-6.200.
(d) Subsurface discharges from wastewater treatment ponds or ash ponds shall, at the property boundary, meet the effluent limitations for subsurface waters of the state under 10 CSR 20-7.015 (7), with appropriate consideration of up-gradient water quality.

14. Sludge and Biosolids Use For Domestic Wastewater Treatment Facilities
(a) Permittee shall comply with the pollutant limitations, monitoring, reporting, and other requirements in accordance with the attached permit Standard Conditions.
(b) If sludge is not removed by a contract hauler, permittee is authorized to land apply biosolids. Permit Standard Conditions, Part III shall apply to the land application of biosolids. Permittee shall notify the department at least 180 days prior to the planned removal of biosolids from the lagoon. The department may require submittal of a biosolids management plan for department review and approval as determined appropriate on a case-by-case basis.

15. Whole Effluent Toxicity (WET) tests will be conducted as follows:

SUMMARY OF WET TESTING FOR THIS PERMIT

OUTFALL	A.E.C. %	FREQUENCY	SAMPLE TYPE	MONTH
002	100%	once/year in year 1 & 4 of the permit term, Report in October	grab	Any

C. SPECIAL CONDITIONS (continued)

15. Whole Effluent Toxicity (WET) test (continued)

(a) Test Schedule and Follow-Up Requirements

(1) Perform a single-dilution test in the months and at the frequency specified above. If the effluent passes the test, do not repeat the test until the next test period.
Submit test results along with complete copies of the test reports as received from the laboratory within 30 calendar days of availability to the WPP, Water Quality Monitoring and Assessment Section, P.O. Box 176, Jefferson City, MO 65102.

(2) If the effluent fails the test, a multiple dilution test shall be performed within 30 calendar days, and biweekly thereafter, until one of the following conditions are met:

(a) THREE CONSECUTIVE MULTIPLE-DILUTION TESTS PASS. No further tests need to be performed until next regularly scheduled test period.
(b) A TOTAL OF THREE MULTIPLE-DILUTION TESTS FAIL.

(3) The permittee shall submit a summary of all test results for the test series along with complete copies of the test reports as received from the laboratory to the WPP, Water Quality Monitoring and Assessment Section, P.O. Box 176, Jefferson City, MO 65102 within 14 calendar days of the third failed test.

(4) Additionally, the following shall apply upon failure of the third test: A toxicity identification evaluation (TIE) or toxicity reduction evaluation (TRE) is automatically triggered. The permittee shall contact WPP, Water Quality Monitoring and Assessment Section to ascertain as to whether a TIE or TRE is appropriate. The permittee shall submit a plan for conducting a TIE or TRE to the Planning Section of the WPP within 60 calendar days of the date of DNR's direction to perform either a TIE or TRE. This plan must be approved by DNR before the TIE or TRE is begun. A schedule for completing the TIE or TRE shall be established in the plan approval.

(5) Upon DNR's approval, the TIE/TRE schedule may be modified if toxicity is intermittent during the TIE/TRE investigations. A revised WET test schedule may be established by DNR for this period.

(6) If a previously completed TIE has clearly identified the cause of toxicity, additional TIEs will not be required as long as effluent characteristics remain essentially unchanged and the permittee is proceeding according to a DNR approved schedule to complete a TRE and reduce toxicity. Regularly scheduled WET testing as required in the permit, without the follow-up requirements, will be required during this period.

(7) All failing test results shall be reported to WPP, Water Quality Monitoring and Assessment Section, P.O. Box 176, Jefferson City, MO 65102 within 14 calendar days of the availability of the results.

(8) When WET test sampling is required to run over one DMR period, each DMR report shall contain information generated during the reporting period.

(9) Submit a concise summary of all test results with the annual report.

C. SPECIAL CONDITIONS (continued)

15. Whole Effluent Toxicity (WET) test (continued)

(b) PASS/FAIL procedure and effluent limitations:

(1) To pass a single-dilution test, mortality observed in the AEC test concentration shall not be significantly different (at the 95% confidence level; p = 0.05) than that observed in the upstream receiving-water control sample. The appropriate statistical tests of significance will be those outlined in the most current USEPA acute toxicity manual or those specified by the MDNR.

(2) To pass a multiple-dilution test:

(a) the computed percent effluent at the edge of the zone of initial dilution, Acceptable Effluent Concentration (AEC), must be less than three-tenths (0.3) of the LC_{50} concentration for the most sensitive of the test organisms; or,
(b) all dilutions equal to or greater than the AEC must be nontoxic. Failure of one multiple-dilution test is an effluent limit violation.

(c) Test Conditions

(1) Test Type: Acute Static non-renewal

(2) Test species: Ceriodaphnia dubia and Pimephales promelas (fathead minnow). Organisms used in WET testing shall come from cultures reared for the purpose of conducting toxicity tests and cultured in a manner consistent with the most current USEPA guidelines. All test animals shall be cultured as described in the most current edition of Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.

(3) Test period: 48 hours at the "Acceptable Effluent Concentration" (AEC) specified above.

(4) When dilutions are required, upstream receiving stream water shall be used as dilution water. If upstream water is unavailable or if mortality in the upstream water exceeds 10%, "reconstituted" water will be used as dilution water. Procedures for generating reconstituted water will be supplied by the MDNR upon request.

(5) Single-dilution tests will be run with:
(a) Effluent at the AEC concentration;
(b) 100% receiving-stream water (if available), collected upstream of the outfall at a point beyond any influence of the effluent; and
(c) reconstituted water.

(6) Multiple-dilution tests will be run with:
(a) 100%, 50%, 25%, 12.5%, and 6.25% effluent, unless the AEC is less than 25% effluent, in which case dilutions will be 4 times the AEC, two times the AEC, AEC, 1/2 AEC and 1/4 AEC;
(b) 100% receiving-stream water (if available), collected upstream of the outfall at a point beyond any influence of the effluent; and
(c) reconstituted water.

(7) If reconstituted-water control mortality for a test species exceeds 10%, the entire test will be rerun.

D. SCHEDULE OF COMPLIANCE

1. The company will develop an overall comprehensive plan detailing a strategy to manage the Sulfate Levels at or below a limit of 1000mg/L. This study will be finished within two years (December 31, 2001) which will allow adequate time to sample during all seasons with various rainfall events.

2. The company will implement the plan within one year from that time (December 31, 2002).

3. The company must be in compliance with sulfate limits by December 31, 2003.

SUMMARY OF TEST METHODOLOGY FOR WHOLE-EFFLUENT TOXICITY TESTS

Whole-effluent-toxicity test required in NPDES permits shall use the following test conditions when performing single or multiple dilution methods. Any future changes in methodology will be supplied to the permittee by the Missouri Department of Natural Resources (MDNR). Unless more stringent methods are specified by the DNR,the procedures shall be consistent with the most current edition of Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms,

Test conditions for Ceriodaphnia dubia:

Test duration:	48 h
Temperature:	25 ± 1°C Temperatures shall not deviate by more than 3°C during the test.
Light Quality:	Ambient laboratory illumination
Photoperiod:	16 h light, 8 h dark
Size of test vessel:	30 mL (minimum)
Volume of test solution:	15 mL (minimum)
Age of test organisms:	<24 h old
No. of animals/test vessel:	5
No. of replicates/concentration:	4
No. of organisms/concentration:	20 (minimum)
Feeding regime:	None (feed prior to test)
Aeration:	None
Dilution water:	Upstream receiving water; if no upstream flow, synthetic water modified to reflect effluent hardness.
Endpoint:	Pass/Fail (Statistically significant Mortality when compared to upstream receiving water control or synthetic control if upstream water was not available at $p \leq 0.05$)
Test acceptability criterion:	90% or greater survival in controls

Test conditions for (Pimephales promelas):

Test duration:	48 h
Temperature:	25 ± 1°C Temperatures shall not deviate by more than 3°C during the test.
Light Quality:	Ambient laboratory illumination
Photoperiod:	16 h light/ 8 h dark
Size of test vessel:	250 mL (minimum)
Volume of test solution:	200 mL (minimum)
Age of test organisms:	1-14 days (all same age)
No. of animals/test vessel:	10
No. of replicates/concentration:	4 (minimum) single dilution method 2 (minimum) multiple dilution method
No. of organisms/concentration:	40 (minimum) single dilution method 20 (minimum) multiple dilution method
Feeding regime:	None (feed prior to test)
Aeration:	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min.
Dilution water:	Upstream receiving water; if no upstream flow, synthetic water modified to reflect effluent hardness.
Endpoint:	Pass/Fail (Statistically significant Mortality when compared to upstream receiving water control or synthetic control if upstream water was not available at $p \leq 0.05$)
Test Acceptability criterion:	90% or greater survival in controls